full-length. In the same study, it was proposed that linker binding to the ATPase domain causes a change in the slow step of the ATP hydrolysis reaction by rearranging the ATPase domain to a conformation where ADP release becomes the rate-limiting step. Here, we are investigating the molecular details of the reason of pH dependence and enhanced ATPase activity upon linker interactions with the domain using ATPase domain constructs both in in vitro and in silico. We observed with molecular dynamic simulations significant upshift in the pKa values of Asp194 and Asp201 compared to their expected pKa values as negatively charged residues, and it seems like that protonation states of these residues at different nucleotide-bound forms are important in the linker derived conformational changes leading, speculatively, variations in the Pi and ADP affinities. Our results will be discussed with overall ATPase rate as well as ADP off-rate measurements on DnaK(1-388) and DnaK(1-392) constructs.

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Effects of Neutral, Capped Ends Versus Charged Ends on the Folding of the Trp-cage Miniprotein

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Charge-charge interactions have a significant impact on the free energy landscape of proteins. The role addition or subtraction of charged groups can play in the stabilization or destabilization of meta-stable non-native states of proteins remains relatively unstudied in detail. Problematically, current experimental methods can only indirectly characterize the non-native states of a protein. All-atom molecular dynamics simulations provide a useful tool for studying the non-native states of a protein but are limited in scale to the smallest proteins. Therefore, in order to study effects of charges while ensuring adequate sampling, we choose to simulate a small, fast-folding model protein, the Trp-cage, with replica-exchange molecular dynamics. As most simulations use a neutral end, capped sequence while experiments tend to use a charged end, zwitterionic variant, we chose to simulate and compare the folding of the Trp-cage both with neutral, capped ends and in zwitterionic form with a positively charged N terminus and a negatively charged C terminus. We find that while the native state of the protein remains relatively unchanged, the equilibration time for the charged end simulation is much longer, suggesting a rougher folding landscape. Analysis suggests the formation of meta-stable states characterized by the possession of non-native charge pairs. Furthermore, we also report an increase in beta-sheet content that cannot be directly explained by the formation of a specific non-native charge pair, indicating the addition of charges plays a much more complex role than the direct creation of non-native charge pairs. We finally use string methods to determine the most probable transition path between non-native meta-stable states and the native state in order to better explain the factors resulting in the increase in equilibration time for the simulation.

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Forced Unfolding of Periplasmic Binding Proteins (PBPS) Follows Kinetic Partitioning

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Periplasmic binding proteins (PBPs) are large two-domain proteins that are present in the periplasmic space of Gram-negative bacteria and they mediate the uptake of small ligands, for example, amino acids and sugars, from the surrounding environment. After their synthesis in the cytoplasm, forced unraveling of PBPs is essential for their translocation into the periplasm. Atomic force microscopy based single-molecule force spectroscopy (SMFS) is a versatile technique to study the mechanical unfolding mechanisms of PBPs such as maltose binding protein (MBP), leucine binding protein (LBP), and ribose binding protein (RBP). On mechanical stretching, MBP follows a kinetic partitioning between two-state and three-state unfolding pathways with 38% of molecules taking the two-state pathway. The flux through the two-state pathway further decreases to 21% upon binding to maltose (1). The unfolding mechanism of LBP is much more complex than MBP. LBP also follows two-state pathway and three-state pathways during the mechanical unfolding; the three-state pathways are more diverse in nature suggesting that LBP takes multiple three-state pathways during mechanical unfolding. Leucine binding influences the unfolding flux more towards a two-state pathway, increasing it from 38% to 65% (2). Similar studies on RBP show that it also follows kinetic partitioning during mechanical unfolding but the percentage of molecules taking the two-state pathway is very high (~85%). These single-molecules studies on PBPs reveal that kinetic partitioning seems to be a general feature for their mechanical unfolding and ligand binding further modulates the unfolding propensity through these pathways.

1. Aggarwal V, Kulothungan SR, Balamurali MM, Saranya SR, Varadarajan R, and Ainavarapu SRK, *J. Biol. Chem.* **2011**, *286*, pp. 28056-28065.
2. Kotamarthi HC, Sharma R, Narayan S, Ray S, and Ainavarapu SRK, *J. Am. Chem. Soc.***2013**, *135,* pp. 14768-14774.

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Impaired-UPS can be Compensated by Activation of Autophagy in Neurodegenerative Diseases

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The ubiquitin-proteasome system (UPS) is a dynamic cellular pathway involved in the deaggregation of misfolded proteins through proteasome degradation. Impaired-UPS function is frequently observed in patients afflicted with neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and Huntington's disease. Increased oxidative stress has long been implicated with the pathogenesis of impaired-UPS function by promoting protein misfolding and the subsequent protein aggregate formation. Autophagy, a bulk lysosomal degradative process, mediates the clearance of toxic cellular constituents and protein aggregates via autophagosome formation and increasing evidence suggests an association between neurodegenerative disorders and defective autophagy. Therefore, it is crucial to understand the role of oxidative stress in impaired-UPS function and the sub-cellular source of oxidative stress. In addition, understanding the mechanism(s) by which autophaghy regulates UPS function will will allow us to develop novel therapeutics for neurodegenerative diseases. Here we demonstrated that rotenone activated nicotinamide adenine dinucleotide phosphatase (NADPH oxidase or Nox2) and Nox2-dependent oxidative stress resulted in impaired UPS machinery. Src kinase was persistently activated by Nox2-dependent superoxide production, which resulted in further Nox2 activation via $p47^{phox}$ phosphorylation and impaired autophagy by activating the autophagy repressor mTOR through PI3K/Akt phosphorylation. Inhibition of Nox2 or Src kinase mitigated excess oxidative stress, which induced autophagy and rescued UPS function. Our data highlight NADPH oxidase and Src kinase as possible autophagy modulators and potential therapeutic targets for common neurodegenerative diseases.

3396-Pos Board B124

Crowding Effects on the Temperature and Pressure Dependent Structure, Stability and Folding Kinetics of S*Taphylococcal Nuclease*

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FT-IR spectroscopic, small-angle X-ray scattering (SAXS) and calorimetric measurements have been applied to explore the effect of the macromolecular crowder agent Ficoll on the temperature- and pressure-dependent stability diagram and folding reaction of the monomeric protein Staphylococcal Nuclease (SNase). Additionally, we compare the experimental data with theoretical predictions. Exploring the crowding effect on the pressure-induced unfolding of proteins provides insights in Protein stability and folding under cell-like densely packed conditions. Complementary SAXS measurements were carried out to explore the suitability of the macromolecular crowder agent over a wide range of temperatures and pressures. We found that both temperature- and pressure-induced equilibrium unfolding of SNase is markedly inhibited in 30 wt% Ficoll solutions. The structure of the unfolded state ensemble does not seem to be strongly influenced in the presence of the crowder, however. For comparison, self-crowding effects have been found to become important at SNase concentrations above 10 wt%, only. In contrast to the common notion that macromolecular crowding increases the rate of Protein folding, our kinetic results show that the folding rate of SNase in fact decreases markedly in the presence of Ficoll. These results indicate that besides the commonly encountered excluded volume effect, other factors need to be considered when assessing confinement effects on protein folding kinetics. Among those, crowder-induced viscosity changes seem to be most prominent.

3397-Pos Board B125

Nanosecond Dynamics of Calmodulin and Ribosome-Bound Nascent Chains Studied by Time-Resolved Fluorescence Anisotropy

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We report a time resolved fluorescence anisotropy (TRA) study of ribosome-bound nascent chains (RNCs) of Calmodulin (CaM), a prototypical member